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Oniscus asellus (Isopoda) as a participant in food webs with increasing complexity in the decomposition of sweet chestnut leaf litter produced in ambient air and in CO_2 enriched atmosphere.

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Summary : Leaves were collected from chestnut trees grown under ambient atmosphere or a double CO_2 concentration (700 ppm). The leaves produced in CO_2 enriched atmosphere showed a lower nitrogen concentration and a higher C/N ratio than those grown in ambient air. Experiments were conducted over 24 weeks to study the decomposition of these two litter qualities. Experimental units received treated (CO_2 enriched) or control (produced in ambient air) cut and sterilized leaves. Then, animal groups with increasing complexity were added to the litter (microflora + Protozoa ; + nematodes ; + Collembola ; + Isopoda). Every two weeks, the CO_2 release was measured and the litter was leached. Analyses were performed on the leachates : pH, total nitrogen, dissolved and particulate carbon, inorganic nitrogen (NH_4^+ and NO_3^-) and biological counts (protozoa, nematodes, rotifera).

For most of the parameters measured, two periods appeared. The first one (about 2-3 months) was dominated by the litter quality effect (C/N ratio), the faunal effect was not yet detectable ; the decomposition rate of treated leaves was lowered. During the second period the decomposition pathways were modified by faunal interactions. The food web effect was all the more evident as the complexity in the animal groups increased. For leached carbon and nitrogen and for pH the highest differences between the two litter types were observed in systems with isopoda. In all the systems the highest nitrogen losses were observed in the units with Isopoda. In the treated litter compared with the control litter the presence of diversified animal groups enhanced the CO_2 release by 30% while in poor faunal food webs it is reduced by 60%.

Bleaching as an indicator of white-rot fungi proliferation, appeared during the late decomposition stages, and all the earlier where the animal groups were more complex, first in the systems with Isopoda, then in the systems with Collembola, later in the systems with nematodes, and only in the treated leaves. Bleaching never appeared in the microflora + Protozoa units. This invasion by fungi was concomitant with a decreasing pH. The biological and chemical interactions favoured by the fragmentation of the litter by animals, led to a modification in CO_2 release, in the leached carbon and in the composition of the decomposers community, increasing the microbial activity. Explanations on the invasion and activity of white-rot fungi are proposed with explanatory remarks about competition or/and chemical inhibition.

Introduction

The carbon dioxide content of the atmosphere has increased during the past century. If the phenomenon continues, the concentration of this gas might double around the middle of the next century. The response of terrestrial ecosystems to higher CO_2 levels remains uncertain (TANS *et al.*, 1990). On land, forests are the largest sources and sinks influencing CO_2 fluxes, but the effects of a doubling of CO_2 concentration on plants are not fully understood. The main results obtained for some species of trees show a change in leaf structure (THOMAS and HARVEY, 1983 ; RADOGLOV and JARVIS, 1990), an increase of the tissue density (mass unit area -1) of the leaves (LEMON, 1983), and an increase in C/N ratio of the tissues resulting from dilution of available nitrogen by increased assimilation (EAMUS and JARVIS, 1989). Such changes in C/N ratio may lead to a reduction of the decomposition rates of the plant material and to modifications of the nutrients availability (LUXMORE, 1981).

The initial C/N ratio, the initial lignin and nitrogen contents or lignin/N ratio are quality factors of the leaf litter (MEENTEMEYER, 1978 ; BERG *et al.*, 1982 ; MELILLO *et al.*, 1982 ; TAYLOR *et al.*, 1989) considered as good predictors of plant litter decomposition dynamics.

The role of the soil fauna appears at different levels in the litter decomposition (ANDERSON, 1973, 1988 ; DOUCE and CROSSLEY, 1982 ; HASSAL *et al.*, 1986 ; HUHTA *et al.*, 1988 ; INESON *et al.*, 1982 ; REDDY and VENKATAIAH, 1989 ; SEASTEDT, 1984 ; SETÄLÄ *et al.*, 1988 ; WILLIAMS and GRIFFITHS, 1989), but the quality of the litter is a critical factor in determining grazing by animals. Therefore, differences in decomposition rates among types of leaf litter may lead to a range of communities with different trophic interactions between the soil fauna and microorganisms (HERLITZIUS, 1983).

In the present study trees were grown under natural and enriched CO_2 concentrations. The experiment was set up to determine (1) the effects of the modification in the litter quality and (2) the influence of interacting groups of soil fauna (an isopod was used) on the mineralization and leaching of carbon and nitrogen.

Material and Methods

Two year old chestnut trees were grown during two additional years under ambient (350±20 ppm) and enriched (700±37 ppm) CO_2 concentrations, in two naturally lit growth chambers located outdoors on the University Campus at Orsay (48°N 2°E) in the South of Paris (MOUSSEAU and ENOCH, 1989). The leaves were collected by MOUSSEAU after the fall in the autumn of the second year for use in the experiments.

Leaf litter from both treatments were cut into $1cm^2$ pieces excluding the main ribs. These pieces of leaves were then washed and sterilized (COUTEAUX *et al.*, 1990).

Twenty-four replicate experimental units received 4.5 g of air-dried sterile leaf pieces in its upper parts ; twelve units, called T units with the CO_2 enriched treated leaves and twelve units, called C units, with control leaves. Sterile demineralized water (150ml per chamber) was added to the underplugged upper chambers to moisten the leaves. After 24 hours, the plug was removed and the water was collected in the lower part of the units with a vacuum pump (275 mBar for 5 minutes). This time was designated as To. All the T and C units were inoculated with 1ml of a suspension of microflora and protozoa prepared by incubating fresh chestnut litter in demineralized water for a few days. For details of the experimental procedures, see COUTEAUX *et al.* (1990).

Table 1 : Designation of the experimental units, n = 3 for each unit
(after Coûteaux *et al.*, 1990)

	Microflora+ Protozoa	Microflora+ Protozoa+ Nematodes	Microflora+ Protozoa+ Nematodes+ Collembola	Microflora+ Protozoa+ Nematodes+ Collembola+ Isopoda
C units Control litter	P-C	NP-C	CNP-C	ICNP-C
T units Treated litter	P-T	NP-T	CNP-T	ICNP-T

On day 19, 350 nematodes of mixed species extracted from a chestnut forest soil, were introduced in 9 of both T and C units (tab. 1). After one month, 20 *Folsomia candida* were added in 6C and 6T units, and 3 *Oniscus asellus* were introduced into 3T and 3C units. The choice of *O. asellus* was justified by its resistance to the experimental conditions. The animals were collected in a deciduous forest in the south of Paris just before the beginning of the experiments and maintained on a diet of a decomposing chestnut leaves some days before. At each sampling interval, dead *O. asellus* were removed and replaced.

Experiments were conducted over 24 weeks. Every two weeks the CO_2 traps (40 ml of 0,5 M NaOH) were changed, the litter was leached and the units were weighed before and after each leaching. Analyses were performed on the leachates : pH, dissolved and particulate carbon, total nitrogen, inorganic nitrogen (NH_4^+, NO_3^-) and biological counts (protozoa, nematodes, rotifera) (COUTEAUX *et al.*, 1990). Statistical analysis were performed on the data : Newmans-Keuls tests were used for comparisons of means, a two-way and a three-way ANOVA were used to analyse the different factors (time, litter quality, complexity of the food webs, interactions).

Results

The carbon and nitrogen contents of leaves were measured before and after washing and sterilizing. The growth of the trees in CO_2 enriched atmosphere did not change the carbon content of the leaves but reduced drastically the nitrogen concentration, giving C/N ratio values of 40 and 75 for C and T material respectively (COUTEAUX *et al.*, 1990).

Detailed results on water content, fresh and dry weight of the leaves, biological counts, pH of the leaching water, carbon and nitrogen loss were given in COUTEAUX *et al.* (1990). In this paper we considered only the main points of interest which seem emerged from the presence of Isopoda in the more complex food webs.

The highest differences of pH of the leaching water from the two litter types were observed in ICNP units. In ICNP-T leachates the pH decreased significantly to between 4 and 5, whereas in the ICNP-C, the pH values increased slowly to 6-7 over 14 weeks. The litter quality factor was always highly significant ($P < 0,001$) while the inoculum appeared to be significant only during the last two months. Significant differences were observed between T and C leachates for several factors (time, litter quality, inoculum diversity and the interaction litter quality x inoculum diversity). No differences related to the inoculum complexity appeared in C leachates, but in T units although no differences were observed between P-T and NP-T, there were significant differences between CNP-T and ICNP-T units.

Table 2 : Loss in carbon at the end of the experiment (% of the initial carbon). A : loss in carbon through mineralization. B : loss of total carbon (dissolved and particulate) in leachates. C : total carbon loss (leached and CO_2-C).

		A	B	C
T treated leaves	P	10	9	19
	NP	20	10	30
	CNP	26	10	36
	ICNP	31	11	42
C control leaves	P	24	7	31
	NP	23	7	30
	CNP	23	7	30
	ICNP	24	8	32

Loss of carbon at the end of the experiment is presented in table 2. The highest loss was observed in ICNP units. In all the systems inoculated with more complex animal groups, the carbon loss in T units was largely higher than in C units.

Two periods of carbon loss appeared. During the first period (2-3 months), the litter quality was the only significant factor, then during the second period this effect was progressively enhanced by the emergence of the inoculum diversity factor (COUTEAUX *et al.*, 1990). For example, the cumulative respiration curves (fig. 1) clearly showed the progressive changes with time as complexity of the animal groups in the inoculum increased.

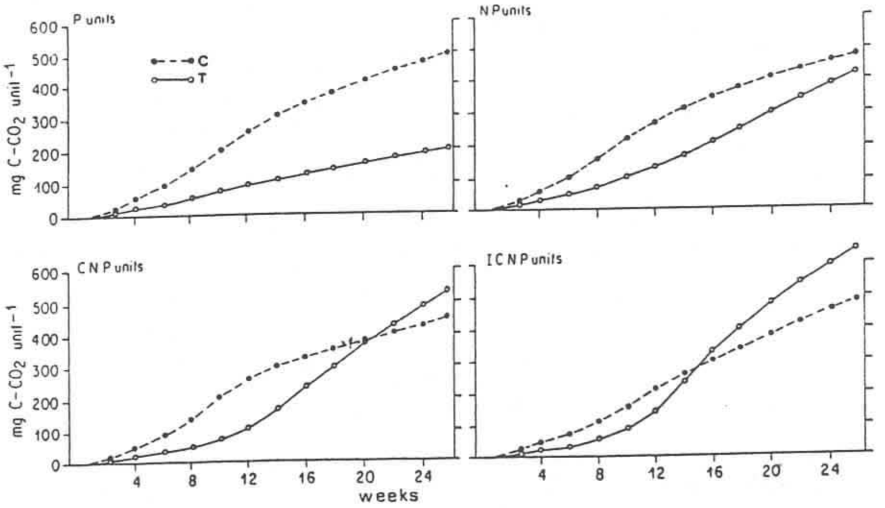

Figure 1 : Cumulative respiration curves (after Coûteaux *et al.*, 1990)

At the end of the experiment the combined effects of litter quality and inoculum diversity led to a higher respiration rate and carbon leaching and to their rise in the T units with the increasing complexity of the food webs (tab. 3).

Table 3 : Some parameters at the end of the experiment (n =3)
(after Coûteaux *et al.*, 1990)

	Cumulated C-CO2 mg unit^{-1}	Cumulated leached C mg unit^{-1}	Cumulated lached mineral N µg unit^{-1}	Cumulated leached N mg unit^{-1}
CO2-enriched litter (T)				
P	196,39±17,05	190,03±7,66	266,65±28,62	2,91±0,57
NP	411,15±78,44	202,14±11,91	320,00±49,05	2,91±0,14
CNP	535,62±132,00	213,08±13,97	264,38±31,07	2,77±0,58
ICNP	643,20±110,40	235,91±15,92	319,49±25,79	3,84±0,7
Control litter (C)				
P	491,03±140,97	153,12±10,33	289,20±18,66	4,15±0,39
NP	469,69±82,30	156,41±10,39	283,95±21,58	4,49±0,61
CNP	450,43±36,82	175,63±24,10	330,06±61,62	4,19±0,30
ICNP	482,90±9,35	177,78±9,15	317,08±93,30	6,03±1,95

Thus in the treated litter compared with the control litter, carbon mineralization is enhanced by 30% in the more complex food webs while it is reduced by 60% in the less complex systems. The cumulative leached nitrogen was generally higher in the C units (tab. 3, fig. 2).

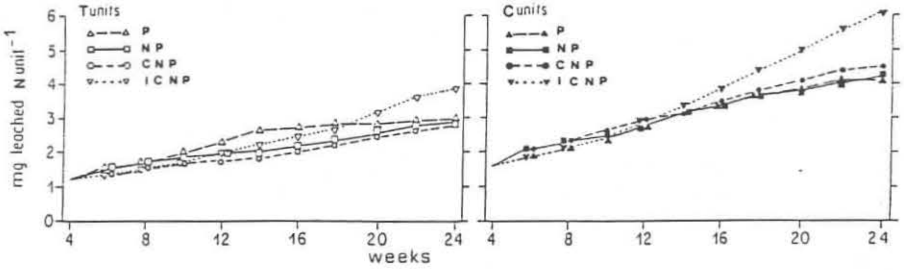

Figure 2 : Cumulative leached nitrogen (mg unit^{-1}) versus time (after Coûteaux *et al.*, 1990).

Nevertheless, since the nitrogen content of the initial litter was lower in the T units the loss, expressed in proportion to initial nitrogen were inverted (tab. 4).

Table 4 : Loss of nitrogen expressed in proportion to the initial nitrogen (%).

	Treated	Control
N NP CNP	11	7
ICNP	14	10

The trend with time was downwards except in the units with Isopoda where the highest nitrogen losses were observed. The nitrogen leaching seems to be controlled by the same factors (litter quality and complexity of the animal groups) than the carbon, but in a reverse way (COUTEAUX *et al.*, 1990).

During the late decomposition stages, bleaching appeared, only in the treated leaves, first in the systems with Isopoda, then in the systems with Collembola, later in the systems with nematodes. It never appeared in the microflora + Protozoa units. Bleaching was a sign of the white-rot fungi growth (TOUTAIN, 1987). White-rot fungi are able to use lignin and aromatic polymer-proteins complexes as a nutrient substrate. In the T units where bleaching appeared progressively, the microbial species composition became different than in the C units (COUTEAUX *et al.*, 1990). This white-rot fungi extension which appeared earlier where the animal groups were more complex was associated with a decrease in pH. The development of white-rot fungi was also concomitant with a modification in the CO_2 release and in the leached carbon.

Discussion

For most of the parameters measured in the experiment, two time periods can be distinguished. The first period was dominated by the litter quality (C/N ratio) effect ; the decomposition rate of treated leaves was lowered, as would be expected for plant material with low nitrogen concentration. The faunal effect was not detectable because it takes some time to develop. During the second period the food web effect was all the more evident as the complexity in the animal groups increased. The decomposition pathways were modified by faunal interactions.

The white-rot fungi extension appeared during the late decomposition stages and only in the systems with high C/N leaves (except NP-T). The modifications in the CO2 release and in the leached carbon indicate an enhanced microbial activity in T units : the maximum CO2 release appeared during this period and was higher when the faunal inoculum was more diverse. One explanation of the invasion of only the poor litter by the white-rot fungi is that the fungal activity was probably stimulated by animal activity, particularly the increase in breakdown of the litter. The same effect occured in the C units, but the higher availability in nitrogen allowed, as is suggested by the microfaunal populations (COUTEAUX *et al.*, 1990) the growth of a more diverse microflora thereby eliminating the white-rot fungi which are not very competitive. At the end of the initial decomposition stages the calculated C/N ratio of the litter were 31 for C and 75 T units. Thus, the differences in nitrogen concentrations have not yet been modified. The initial quality of the litter induced an essential difference in the biological interactions, switching an existing competitive relationship by selective grazing. So, the interactions between fauna and microflora led to different communities with different functions. Another explanation might be found in biochemical interactions between ammonium or amino acids and phenolic polymers or lignolytic enzymes, as suggested by results of KEYSER *et al.* (1978), NÖMMIK and VAHTRAS (1982), BERG *et al.* (1982), BERG (1986). A high initial decomposition rate in litter with high nitrogen level may promote chemical changes that may enhance the resistance of lignin. In the present experiment the litter treated with low nitrogen content may have favoured the development of organisms which are able to degrade resistant compounds during the late decomposition stages. This degradation was enhanced by the animal contribution.

In all the systems the highest nitrogen losses were observed in the units with isopoda. After the introduction of the animals, the units with Isopoda showed a substantial NH_4^+ production which could have been due to excretion by Isopoda (HARTENSTEIN, 1968 ; WIESER and SCHWEIZER, 1970 ; WIESER, 1984) during a period when microbial activity, and thus the immobilization of nitrogen in the microflora, were low. GUNNARSSON and TUNLID (1986), MOCQUARD *et al.* (1987), JAMBU *et al.* (1988) have observed an increase of nitrogen in the fecale pellets of *Oniscus asellus* which might explain the increase of organic nitrogen in the leachates because the fecale pellets can be carried away in the leachates.

Even if particulate carbon is more important in C litter, in the present experiment it cannot be poved whether isopods have prefered one or other of the litter qualities, although RUSHTON and HASSAL (1983) have shown that this factor might occured. It is clear that the presence of isopods has modified the interactions between the microbial communities and has allowed, in both the poor and the rich nitrogen litter, the development of different communities.

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